PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 16033-WO-03	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/IL2004/000461	International filing date *(day/month/year)* 31.05.2004	Priority date *(day/month/year)* 02.06.2003
International Patent Classification (IPC) or national classification and IPC C07K14/47, A61K38/00		
Applicant HADASIT MEDICAL RESEARCH SERVICES & DEVEL... et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the International application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31.03.2005	07.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Young, C Telephone No. +49 89 2399-7877

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IL2004/000461

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-37 as originally filed

   Sequence listings part of the description, Pages

   1-10 as originally filed

   Claims, Numbers

   1-22 filed with the demand

   Drawings, Sheets

   1-8 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-22
	No:	Claims	1
Inventive step (IS)	Yes:	Claims	
	No:	Claims	2-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents/:

D1: KASOF G M ET AL: "Livin, a novel inhibitor of apoptosis protein family member" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 276, no. 5, 2 February 2001 (2001-02-02), pages 3238-3246, XP002973441 ISSN: 0021-9258

D2: LIN JIING-HUEY ET AL: "KIAP, a novel member of the inhibitor of apoptosis protein family" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 279, no. 3, 29 December 2000 (2000-12-29), pages 820-831, XP002296037 ISSN: 0006-291X

D3: VUCIC DOMAGOJ ET AL: "ML-IAP, a novel inhibitor of apoptosis that is preferentially expressed in human melanomas" CURRENT BIOLOGY, vol. 10, no. 21, 2 November 2000 (2000-11-02), pages 1359-1366, XP002296038 ISSN: 0960-9822

D4: ASHHAB^A Y ET AL: "Two splicing variants of a new inhibitor of apoptosis gene with different biological properties and tissue distribution pattern" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 495, no. 1-2, 20 April 2001 (2001-04-20), pages 56-60, XP004235763 ISSN: 0014-5793

D5: DATABASE EMBL BIR7 sequence 28 February 2003 (2003-02-28), XP002296040 retrieved from EBI Database accession no. Q96CA5

D6: DEVERAUX QUINN L ET AL: "Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 18, no. 19, 1 October 1999 (1999-10-01), pages 5242-5251, XP002170857 ISSN: 0261-4189

Novelty, Article 33 (2) PCT

Claim 1 recites p30 Livin alpha and p28 livin beta in the absence of reference to SEQ ID Nos this definition is considered to be internal nomenclature and is objected to under

Article 6 PCT, as a consequence the claimed subject-matter is indistinguishable from the prior art and a novelty objection is raised.

D1 to D5 disclose livin and derived peptides comprising SEQ ID Nos 1 and 2 of the present application. Each disclosure deals with livin as an inhibitor of apoptosis and in no way anticipates a pro-apoptotic function for this peptide. Thus, although the structural features of claims 2 and 3, as presently formulated, are identical with the sequences of D1-D5 the feature pro-apoptotic renders them novel.

Claim 4 and 5 are most certainly novel over the cited art as these relate to the core of the alleged invention namely to two fragments of livin previously not identified.

Claim 6 to 17 relate to pharmaceutical compositions for inducing or enhancing apoptosis using a livin derived peptide. Again the alleged pro-apoptotic activity or in this instance uses for enhancing or inducing apoptosis make these claims novel. By way of analogy claims 18-22 are deemed also to be novel.

Inventive step, Article 33 (3) PCT

The present application relates to fragments of alpha and beta livin allegedly possessing pro-apoptotic activity. The closest prior art is identified as D6. D6 discloses a related family member, XIAP shown to possess two discreet domains governing pro and anti apoptotic activity independently.

The objective problem is defined as;
"The provision of pro-apoptotic fragments of the apoptosis inhibitor livin"

D6 indicates, see pg 5247-5249, that other apoptosis inhibitors of the family IAP may have similar domain architecture to XIAP for which the authors demonstrate a surprising pro-apoptotic activity. Thus, in light of D6 it is not surprising *per se* that an IAP peptide fragment is pro-apoptotic, in this case livin. However, the exact structural features of this fragments giving rise to the pro-apoptotic activity are not derivable from D6.
No guarantee exits that the theory laid out in D6 is indeed correct. It is the opinion of this

authority given the conservative nature of the skilled person that there is no expectancy of success despite the prompting of the closest prior art to formulate that problem defined above.
In short were the application to relate specifically to peptides as claimed in claims 4 and 5 i.e. structurally distinct sequences with regard full length, inventive step requirements would be met. However, at present the application relates to sequences being comprised, and/or substantially similar or even analogous to SEQ ID 1 and 2. This does not delineat itself from the prior art and would not even expect to possess the alleged pro-apoptotic activity. Consequently as presently claimed the objective problem is not shown to be solved. Given the teachings of the cited art there exists reasons to doubt that the full length peptides are indeed pro-apoptotic. As such inventive step is not acknowledged. In the event that the Applicant asserts that wildtype sequences are also pro-apoptotic D1-D5 will automatically become novelty destroying as these sequences would then possess this function intrinsically.

In short restriction to the fragments shown to have pro-apoptotic activity with the appropriate functional features would in principle overcome the above objections.

Note the use of terms analogues, derivatives and internal nomenclature such as p28 and p30 introduce unnecessary ambiguity and are objected to under Article 6 PCT.

New Claims:

1. A Livin-derived peptide selected from any one of p30-Livin α and p28-Livin β.

2. A peptide as defined in claim 5, wherein said p30-Livin α peptide comprises the sequence substantially as defined in SEQ. ID. NO.1, or functional analogues, derivatives or fragments thereof having pro-apoptotic activity.

3. A peptide as defined in claim 5, wherein said p28-Livin β peptide comprises the sequence substantially as defined in SEQ. ID. NO.2, or functional analogues, derivatives or fragments thereof having pro-apoptotic activity.

4. A peptide as defined in claim 5, wherein said p30-Livin α has the amino acid sequence as defined in SEQ. ID.NO.1.

5. A peptide as defined in claim 5, wherein said p28-Livin β has the amino acid sequence as defined in SEQ. ID.NO.2.

6. A pharmaceutical composition for inducing and/or enhancing apoptosis, comprising as active ingredient at least one peptide as defined in any one of claims 5 to 9.

7. A pharmaceutical composition as defined in claim 10 for enhancing apoptosis, wherein said apoptosis is induced by a treatment or agent selected from any one of etoposide, anti-CD95/Fas, TNFα and staurosporine.

8. A pharmaceutical composition as defined in claim 10, for inducing programmed cell death.

9. A pharmaceutical composition as defined in claim 12, for inducing programmed cell death of malignant cells.

10. Use of a peptide as defined in any one of claims 5 to 9, as an agent for the induction of apoptosis.

11. Use of a peptide as defined in any one of claims 5 to 9, as an agent for the enhancement of apoptosis.

12. Use of a peptide as defined in any one of claims 5 to 9, as an agent for the induction of programmed cell death.

13. Use of a peptide as defined in any one of claims 5 to 9, as an agent for the induction of programmed cell death in malignant cells.

14. Use of a peptide as defined in any one of claims 5 to 9, as an agent for enhancing the sensitivity of cells to death-inducing treatments or agents.

15. The use as defined in claim 18, wherein said cells are malignant cells.

16. Use of a pharmaceutical composition as defined in any one of claims 10 to 13, as an agent for enhancing the sensitivity of cells to death-inducing treatments or agents.

17. The use as defined in any one of claims 18 to 20, wherein said death-inducing treatments or agents are selected from any one of etoposide, anti-CD95/Fas, TNFα and staurosporine.

18. The use as defined in any one of claims 18 to 21, wherein said cells are malignant cells.

19. Method of preparation of a pharmaceutical composition for the induction of apoptosis, comprising the step of admixing any one of the peptides as defined in claims 5 to 9, with a pharmaceutically acceptable adjuvant, carrier or diluent, and optionally with at least one additional active agent.

20. Method of enhancing the sensitivity of cells to death-inducing treatments or agents, comprising the steps of:

(a) Introducing a Livin-derived peptide as defined in any one of claims 5 to 9 into a cell; and

(b) Treating said cell with death-inducing agents or treatments.

21. The method as defined in claim 24, wherein said cells are malignant cells.

22. Use of the pharmaceutical composition as defined in any one of claims 10 to 13 for the treatment of cancer.